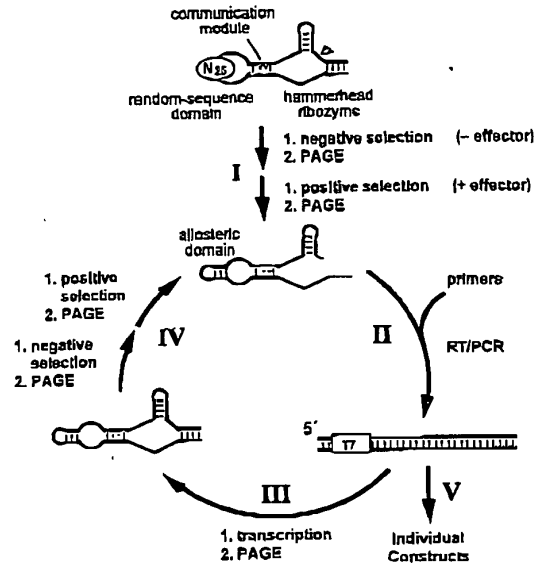

Figure 1. Scheme for the "allosteric selection" of ribozymes that are activated by specific effector molecules. Precursor RNAs are (I) subjected to negative selection in the absence of effector. Uncleaved RNAs are isolated by PAGE, then subjected to positive selection in the presence of a mixture of the four cNMPs. Cleaved RNAs are (II) amplified by RT-PCR to generate double stranded DNA templates. The resulting DNAs are (III) transcribed using bacteriophage T7 RNA polymerase (T7 RNAP) to generate a new population of RNA molecules that are (IV) subjected to the next round of negative and positive selections. (V) Double-stranded DNAs from the desired rounds of selection are cloned and sequenced for further analysis. The boxed T7 represents a double-stranded promoter sequence for T7 RNAP.

RESULTS AND DISSCUSSION

Using the selection scheme depicted in Fig. 1, we conducted repeated rounds of negative and positive for *in vitro* selection for allosteric ribozymes sensitive to cAMP, cGMP, cCMP, and cUMP. In generation 18, 20, and 23, specific responses for cGMP, cCMP, and cAMP, respectively, were observed. However, specific cleavage with cUMP was not observed even after 28 rounds of *in vitro* selection. The effector specificities of the representative clones cGMP-1, cCMP-1, and cAMP-1 were determined as described in Fig. 2. Isolated clones exhibit a specific allosteric response only when presented with their corresponding cNMP effector. Specifically, the ratio of the observed rate constants in the presence (k_{obs}^{+}) versus the absence (k_{obs}^{-}) of the corresponding effector for the cGMP-1, cCMP-1, and cAMP-1 clones are 510, 70, and 59, respectively. These values reflect the rate enhancements that are induced by the cNMP effectors.

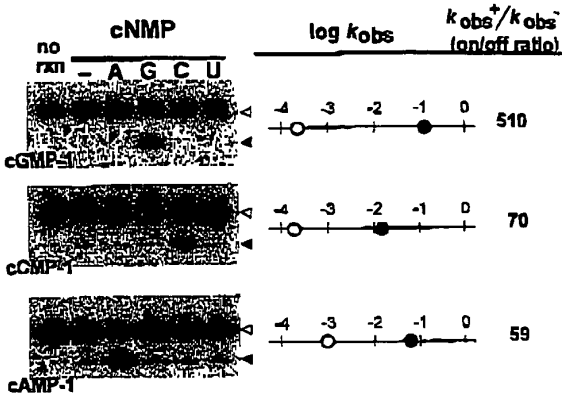

Figure 2. Selective activation of representative allosteric ribozymes by cNMPs. Internally ^{32}P-labeled RNAs that are sensitive to cGMP, cCMP and cAMP (as identified) were incubated for 15 min in the absence of effector (–) or in the presence of 500 μM of the nucleoside 3′,5′-cyclic monophosphates A, G, C and U as indicated under the reaction conditions used for *in vitro* selection. Reaction products were separated by denaturing 10% PAGE and the bands were visualized and quantified using a PhosphorImager and ImageQuant software (Molecular Dynamics). Open and filled arrowheads identify the precursor and 5′ cleavage products, respectively. The 3′ cleavage products have greater electrophoretic mobility than the significantly larger precursor RNAs and 5′-cleavage fragments, and therefore are not present on the images.

Using various analogues for the cNMPs, we investigated the requirements for the specific recognition the allosteric effectors. For example, each clone remains inactive in the presence of its corresponding nucleoside 5′-monophosphate or nucleoside 3′-monophosphate analogue of cNMP, indicating that each effector-binding site can distinguish small differences in the chemical structures of their ligand. Also, when cNMP was added into ribozyme reaction, rapid effector-dependent activation was observed instantaneously. These results indicate that allosteric ribozymes can rapidly convert to an active state upon specific recognition of their cognate effector molecule. Further optimization of these allosteric ribozymes could lead to their use as precision biosensor elements or as novel genetic control elements.

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